UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

DATE: June 23, 2011

SUBJECT: **N-Butyl-1,2-Benzisothiazolin-3-One (BBIT):** DERs of Mammalian Toxicity Studies Submitted for the Existing Registration of Vanquish® Technical

PC Code(s): 098951	DP Barcode(s)/No(s): D384564
Decision No.: 442760	Registration No(s).: 1258-1267
Petition No(s).: NA	Regulatory Action: Action Code 570
Risk Assess Type: Single Chemical	Case No(s).: None
TXR No.: 1,003,229	CAS No(s): 4299-07-4
MRID No(s).: 48262204; 48261201	40 CFR: N/A

FROM: Jenny J. Tao, Senior Toxicologist
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (AD) (7510P)

TO: Marshall Swindell, Product Manager
Dennis Edwards, Chief
Regulatory Management Branch I
AD

THRU: Jonathan Chen, Ph.D., Senior Toxicologist (peer reviewer)

And

Nader Elkassabany, Ph.D., Chief
RASSB
AD

The registrant, Arch Chemical, Inc., submitted the following mammalian toxicity studies and stated in the transmittal letter that "These studies were originally performed for the European Biocides Products Directive and is being sent to the Agency in further support of our registrations containing N-butyl-1,2-benzisothiazolin-3-one." Vanquish® Technical, a manufacturing use product (MUP), is an existing conditional registration and contains 99.2% of N-butyl-1,2-benzisothiazolin-3-one as the active ingredient.

This memo includes executive summary of the reviews and DERs of the submitted studies. The submitted toxicity studies include:

MRID 48262204: A 90-day oral (capsule) toxicity study of N-butyl benzisothiazolin-3-one in Beagle dogs.

MRID 48261201: A dietary two-generation reproductive toxicity study of N-butyl benzisothiazolin-3-one in rats.

(1) Subchronic Oral Toxicity (Feeding – Dog)

Mertens, J. (2007) A 90-day oral (capsule) toxicity study of N-Butyl Benzisothiazolin-3-One in beagle dogs. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Study No.: WIL-544002, 19 February 2007. MRID 48262204. Unpublished.

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 48262204), N-butyl-1,2-benzisothiazolin-3-one (99.4% a.i.; Lot No. 6180) in the vehicle, corn oil, was administered by gelatin capsule, once daily, to four beagle dogs/sex/dose group at doses of 0, 25, 75 or 250 mg/kg/day for at least 90 days. The high dose was reduced to 200 mg/kg/day beginning on Day 10 due to one female who was sacrificed *in extremis* at 250 mg/kg/day.

No adverse, treatment-related effects were observed on ophthalmoscopic examinations, urinalysis, organ weights or gross or microscopic pathology.

Mortality was observed at high-dose level. One female was sacrificed *in extremis* due to abnormal excreta, dermal atonia, emesis, excessive salivation, hypoactivity and thinness. Treatment-related clinical observations consisted of emesis, diarrhea, soft feces and some mucoid feces in the mid- and high-dose males and females. Treatment-related effects on body weights were observed at high-dose level in both sexes. Males at this dose actually lost weight ($p<0.05$) during Weeks 0-1 and 0-2. Cumulative body weight gains in the males at this dose remained decreased compared to controls throughout the study, and attained statistical significance ($p<0.05$). Overall (Weeks 0-13) body weight gain in high-dose males was decreased compared to controls. As a result of these decreases, body weights were decreased (non-significant) in these males throughout the study. Treatment-related effects in the high-dose females were limited to decreases (non-significant) in body weight during Weeks 1 and 2. All other statistically significant differences from controls in body weight or body weight gain were minor, transient, and/or unrelated to dose. Red blood cell parameters (red cell count, hemoglobin, and hematocrit) were decreased in males and females at both testing intervals (non-significant). This change was not dose-dependent, but the high-dose groups consistently showed the greatest decrease, while higher platelet counts were found in this dose group. A dose-response decrease in serum albumin and total protein concentrations was observed in the high-dose males and females ($p\leq0.01$). Serum albumin concentration was decreased in the low-dose males and total protein concentration was decreased ($p\leq0.05$) in the low-dose females. Serum calcium, which is predominantly bound to albumin, was also decreased ($p<0.01$ or NS) in the high-dose males and females, but the effect in females was only observed during week 7. Triglycerides were increased or decreased in all treated males, but this effect was not dose-dependent.

The LOAEL is 75 mg/kg/day, based on treatment-related clinical findings in both sexes and dose-response decreases in albumin (males) and total protein concentrations (females). The NOAEL is 25 mg/kg/day.

This study is classified **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3150; OECD 409) for a subchronic oral toxicity study in dogs.

(2) Reproduction and Fertility Effects Study – Rats

Stump, D.G. (2007) A dietary two-generation reproductive toxicity study of *N*-butyl benzisothiazolin-3-one in rats. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Study No.: WIL-544004, April 16, 2007. MRID 48261201. Unpublished.

EXECUTIVE SUMMARY: In a two-generation reproduction toxicity study (MRID 48261201), *N*-butyl benzisothiazolin-3-one (99.4±1%; Batch # 6180) was administered continuously in the diet to 30 Sprague-Dawley rats/sex/dose group at dietary levels of 0, 300, 600, or 1700 ppm (equivalent to 0/0, 25/27, 49/56, and 141/157 mg/kg/day in males/females, respectively) for two consecutive generations. The P and F1 generation animals were exposed to the test diets for a minimum of 70 days prior to mating. F1 offspring selected to be parents of the F2 generation (30/sex/dose group) were fed the same test diet concentrations as their parents beginning on PND 21. The F2 offspring were terminated after weaning.

All P generation parents survived to scheduled sacrifice. Three F1 adults were found dead or sacrificed during the study; however, none of these mortalities were related to treatment. All other F1 adults survived to scheduled sacrifice.

In the P generation at high-dose level, overall pre-mating (Weeks 0-10) body weight gain was decreased significantly ($p<0.05$) in the females, but was not significantly different from controls in the males. Mean P generation maternal body weights, body weight gains, absolute (g/animal/day) and relative (g/kg/day) food consumption, and food efficiency (%) were unaffected by treatment during gestation.

In the F1 generation at high-dose level, body weights were decreased significantly throughout the pre-mating period (Weeks 18-28) in the males ($p<0.05$) and in the females ($p<0.01$). Overall pre-mating body weight gains were similar to controls at all doses in both sexes. Absolute food consumption was decreased ($p<0.05$) during Weeks 18-19 ($p<0.01$) through 22-23 and 25-26 for males and females, respectively. Relative food consumption was similar to controls for both males and females. Food efficiency was similar to controls for males throughout the pre-mating period but increased ($p<0.05$) during Week 18-19 for females.

Mean F1 generation maternal body weights were decreased at high-dose level during gestation. However, these decreases did not attain statistical significance and there were no significant decreases in body weight gains during gestation. These decreases in body weight were considered to be a continuation of the lower mean body weights noted for these females during pre-mating. Overall body weight gains during gestation were similar to controls at all doses. Mean F1 maternal absolute and relative food consumption and food efficiency were unaffected by treatment during gestation. Overall

body weight gains, mean F1 maternal absolute and relative food consumption, and food efficiency were unaffected by treatment during lactation.

No adverse effects of treatment were noted at low- or mid-dose level in either generation.

The LOAEL for parental toxicity is 1700 ppm (equivalent to 141/157 mg/kg/day in males/females, respectively) based on decreased body weights, body weight gains, and food consumption. The NOAEL is 600 ppm (equivalent to 49/56 mg/kg/day in males/females, respectively).

No adverse clinical signs were observed in the F1 or F2 generation pups from birth to PND 21 at any dose. No treatment-related differences in litter parameters were noted at any dose in either generation. Sexual maturation was unaffected by treatment. There were no treatment-related effects on gross pathology or histopathology.

At high-dose level, body weights were decreased ($p<0.05$) in the F1 generation and the F2 generation on PND 21. Overall (PND 1-21) pup body weight gains were decreased in both males and females of both generations.

In the F2 generation pups, treatment-related decreases ($p<0.05$) in absolute and relative (to body) spleen weights were observed in both sexes on PND 21.

The LOAEL for offspring toxicity is 1700 ppm (equivalent to 141/157 mg/kg/day in males/females, respectively) based on decreased body weights, body weight gains, and spleen weight (F2 pups only). The NOAEL is 600 ppm (equivalent to 49/56 mg/kg/day in males/females, respectively).

No treatment-related effects on the mating, fertility, conception/copulation, or gestation indices were observed in either generation. There were no effects of treatment on pre-coital interval or gestation duration in either generation. No signs of dystocia were noted at any dose. Mean estrous cycle lengths were unaffected by treatment in both generations. No treatment-related effects were observed on P or F1 generation spermatogenesis endpoints (mean testicular and epididymal sperm numbers, sperm production rate, motility, progressive motility, and morphology).

The LOAEL for reproductive toxicity was not observed. The NOAEL is 1700 ppm (equivalent to 141/157 mg/kg/day in males/females, respectively).

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.3800; OECD 416) for a two-generation reproduction study in the rat.

DERs of the submitted studies are attached.

EPA Reviewer: Jenny Tao
Risk Assessment and Science Support Branch, Antimicrobial Division

Signature:
Date 6/21/11

EPA Secondary Reviewer: Jonathan Chen, Ph. D.
Risk Assessment and Science Support Branch, Antimicrobial Division

Signature: Jonathan Chen
Date 06/21/2011

DATA EVALUATION RECORD

STUDY TYPE: Reproduction and Fertility Effects Study - [rat]; OPPTS 870.3800 [§83-4]; OECD 416.

PC CODE: 098951 **DP BARCODE:** D384564

TXR#: 1,003,229

TEST MATERIAL (PURITY): *N*-butyl benzisothiazolin-3-one (99.4±1% a.i.)

SYNONYMS: 2-Butyl-1,2-benzisothiazolin-3(2H)-one; BBIT

CITATION: Stump, D.G. (2007) A dietary two-generation reproductive toxicity study of *N*-butyl benzisothiazolin-3-one in rats. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Study No.: WIL-544004, April 16, 2007. MRID 48261201. Unpublished.

SPONSOR: Arch Chemicals, Inc., 501 Merritt 7, Norwalk, CT

EXECUTIVE SUMMARY: In a two-generation reproduction toxicity study (MRID 48261201), *N*-butyl benzisothiazolin-3-one (99.4±1%; Batch # 6180) was administered continuously in the diet to 30 Sprague-Dawley rats/sex/dose group at dietary levels of 0, 300, 600, or 1700 ppm (equivalent to 0/0, 25/27, 49/56, and 141/157 mg/kg/day in males/females, respectively) for two consecutive generations. The P and F1 generation animals were exposed to the test diets for a minimum of 70 days prior to mating. F1 offspring selected to be parents of the F2 generation (30/sex/dose group) were fed the same test diet concentrations as their parents beginning on PND 21. The F2 offspring were terminated after weaning.

All P generation parents survived to scheduled sacrifice. Three F1 adults were found dead or sacrificed during the study; however, none of these mortalities were related to treatment. All other F1 adults survived to scheduled sacrifice.

In the P generation at high-dose level, overall pre-mating (Weeks 0-10) body weight gain was decreased significantly ($p<0.05$) in the females, but was not significantly different from controls in the males. Mean P generation maternal body weights, body weight gains, absolute

(g/animal/day) and relative (g/kg/day) food consumption, and food efficiency (%) were unaffected by treatment during gestation.

In the F1 generation at high-dose level, body weights were decreased significantly throughout the pre-mating period (Weeks 18-28) in the males ($p<0.05$) and in the females ($p<0.01$). Overall pre-mating body weight gains were similar to controls at all doses in both sexes. Absolute food consumption was decreased ($p<0.05$) during Weeks 18-19 ($p<0.01$) through 22-23 and 25-26 for males and females, respectively. Relative food consumption was similar to controls for both males and females. Food efficiency was similar to controls for males throughout the pre-mating period but increased ($p<0.05$) during Week 18-19 for females.

Mean F1 generation maternal body weights were decreased at high-dose level during gestation. However, these decreases did not attain statistical significance and there were no significant decreases in body weight gains during gestation. These decreases in body weight were considered to be a continuation of the lower mean body weights noted for these females during pre-mating. Overall body weight gains during gestation were similar to controls at all doses. Mean F1 maternal absolute and relative food consumption and food efficiency were unaffected by treatment during gestation. Overall body weight gains, mean F1 maternal absolute and relative food consumption, and food efficiency were unaffected by treatment during lactation.

No adverse effects of treatment were noted at low- or mid-dose level in either generation.

The LOAEL for parental toxicity is 1700 ppm (equivalent to 141/157 mg/kg/day in males/females, respectively) based on decreased body weights, body weight gains, and food consumption. The NOAEL is 600 ppm (equivalent to 49/56 mg/kg/day in males/females, respectively).

No adverse clinical signs were observed in the F1 or F2 generation pups from birth to PND 21 at any dose. No treatment-related differences in litter parameters were noted at any dose in either generation. Sexual maturation was unaffected by treatment. There were no treatment-related effects on gross pathology or histopathology.

At high-dose level, body weights were decreased ($p<0.05$) in the F1 generation and the F2 generation on PND 21. Overall (PND 1-21) pup body weight gains were decreased in both males and females of both generations.

In the F2 generation pups, treatment-related decreases ($p<0.05$) in absolute and relative (to body) spleen weights were observed in both sexes on PND 21.

The LOAEL for offspring toxicity is 1700 ppm (equivalent to 141/157 mg/kg/day in males/females, respectively) based on decreased body weights, body weight gains, and spleen weight (F2 pups only). The NOAEL is 600 ppm (equivalent to 49/56 mg/kg/day in males/females, respectively).

No treatment-related effects on the mating, fertility, conception/copulation, or gestation indices

were observed in either generation. There were no effects of treatment on pre-coital interval or gestation duration in either generation. No signs of dystocia were noted at any dose. Mean estrous cycle lengths were unaffected by treatment in both generations. No treatment-related effects were observed on P or F1 generation spermatogenesis endpoints (mean testicular and epididymal sperm numbers, sperm production rate, motility, progressive motility, and morphology).

The LOAEL for reproductive toxicity was not observed. The NOAEL is 1700 ppm (equivalent to 141/157 mg/kg/day in males/females, respectively).

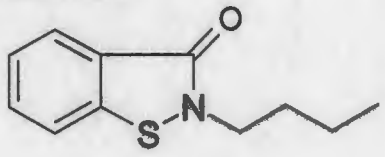
This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements (OPPTS 870.3800; OECD 416) for a two-generation reproduction study in the rat.

COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: *N*-butyl benzisothiazolin-3-one

Description:	Thick, clear, amber liquid
Batch #:	6180
Purity:	99.4±1% a.i.
Compound stability:	The test material was shown to be stable in the diet for up to 15 days at room temperature.
CAS # of TGAI:	4299-07-4
Structure:	

2. Vehicle: Diet

3. Test animals

Species:	Rat
Strain:	Crl:CD(SD)
Age at study initiation:	(P) Approximately 6 weeks
Body weight at initiation of treatment:	(P) Males: 203-250 g; Females: 151-195 g
Source:	Charles River Laboratories, Inc. (Raleigh, NC)
Housing:	During pre-mating, all rats were individually housed in Makrolon type-3 cages with wire-mesh tops. During cohabitation, one male and one female were housed together. Following mating or at the end of the mating period, dams were returned to their cages for the birth and rearing of the litters. On the day of weaning, the dams were separated from their pups and the pups were divided by sex.
Diet:	Certified Rodent LabDiet® 5002 (PMI Nutrition International, LLC), *ad libitum*
Water:	Reverse osmosis-purified water, *ad libitum*.
Environmental conditions:	**Temperature** 22±3°C **Humidity** 50±20% **Air changes** At least 10/hour **Light cycle** 12 hrs light : 12 hrs dark
Acclimation period:	14 days

B. PROCEDURES AND STUDY DESIGN

1. In-life dates: P generation Start: March 7, 2006 End: July 21, 2006
F1 generation Start: June 27, 2006 End: November 21, 2006

2. Mating procedure: Mating was accomplished by co-housing one female with one male for up to 14 consecutive days. Evidence of mating was assessed each morning following pairing

by examining a vaginal smear prepared from each female for the presence of spermatozoa and/or the presence of a vaginal plug. The day on which positive evidence of mating was found was designated as gestation day (GD) 0. Once mating had occurred, the males and females were separated.

3. **Study schedule:** P generation rats (30/sex/dose group) were fed the test diets for a minimum of 70 days prior to mating to produce the F1 litters. Following the mating and gestation periods, dams were allowed to deliver the F1 litters. On post-natal day (PND) 4, litters were standardized to 8 pups/sex/dose group (4/sex/group where possible). The remaining F1 pups were reared until weaning on PND 21. Following weaning, F1 offspring (30/sex/dose group) were randomly selected to be parents of the next generation and were fed the same test diet concentration as their dam for at least 70 days prior to mating to produce the F2 generation. The F2 pups were terminated on PND 21.

4. **Animal assignment:** Before commencement of treatment, the P animals were assigned (stratified by body weight in a block design) to the dose groups shown in Table 1 using a computer-generated randomization procedure. Following weaning on PND 21, F1 offspring (30/sex/dose group) were randomly selected to be parents of the F2 generation.

TABLE 1. Animal assignment [a]

Test group	Dietary conc. (ppm) [b]	Animals/group			
		P Males	P Females	F_1 Males	F_1 Females
Control	0	30	30	30	30
Low	300	30	30	30	30
Mid	600	30	30	30	30
High	1700	30	30	30	30

a Data were obtained from page 36 of the study report.
b Exposure to the test substance was continuous throughout the study.

5. **Dose-selection rationale:** The dietary concentrations listed in Table 1 above were selected based on the results of a previously conducted range-finding reproductive toxicity study in rats (Project No. WIL-544003, 2006). In that study, bred female rats (10/dose) were offered test diets at concentrations of 0, 800, 1300, or 2000 ppm from GD 0 through LD 21. No evidence of maternal toxicity was noted at 800 ppm; however, body weight gains and food consumption were decreased in the 1300 ppm dams during GD 1-4 and in the 2000 ppm dams throughout gestation and lactation. At 2000 ppm, pup mortality was observed, and decreased pup body weight gains were noted at all doses during PND 14-21. Administration of the test diet to F1 weanlings beginning on PND 21 at 2000 ppm resulted in morbidity (hypoactivity, decreased defecation and/or prostration) by PND 25. At 1300 and 2000 ppm, F1 animals displayed decreased body weights and/or food consumption during PND 21-35. Liver weights were increased in the F1 males at 800 ppm and in both sexes at 1300 and 2000 ppm.

6. **Test diet preparation and analysis:** Dietary formulations were prepared weekly by mixing the appropriate amount of test material with a small amount of diet to form a premix. This

premix was then further diluted with diet to achieve the desired dietary concentration. The test diets were stored in bags at room temperature (600 and 1700 ppm) or frozen (300 ppm). Frozen diets were thawed to room temperature prior to presentation to the animals. Stability for up to 15 days at room temperature was verified. Homogeneity (top, middle, and bottom) was determined at all concentrations prior to initiation of dosing. Concentration analyses were determined from dietary preparations prepared during the first 4 weeks of dosing and monthly thereafter.

Results

Homogeneity (% RSD): 1.8-4.4%

Stability (% nominal concentration): 91.3-97.3% after up to 15 days at room temperature

Concentration:

Dietary concentration (ppm)	Mean % nominal
300	88.2-109%
600	91.6-101%
1700	92.6-102%

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the study animals was acceptable.

7. **Dosage administration:** The test material was administered in the diet continuously throughout the study (i.e., P generation adults were fed the test diets *ad libitum* beginning 70 days prior to mating, and the selected F1 adults were fed the same test diet concentrations as their parents beginning on PND 21 for a minimum of 70 days prior to mating.)

C. OBSERVATIONS

1. **Parental animals:** All animals were observed at least twice daily for mortality and clinical signs of toxicity. Detailed physical examinations were performed weekly throughout the study. Females expected to deliver were also observed twice daily during the period of expected parturition and at parturition for dystocia or other difficulties. In the males, body weights were recorded at the initiation of dosing, weekly throughout the study, and prior to scheduled sacrifice. In the females, body weights were recorded at the initiation of dosing and weekly until evidence of copulation was observed. After mating, females were weighed on GD 0, 4, 7, 11, 14, 17, and 20. Dams which littered were weighed on lactation days (LD) 1, 4, 7, 14, and 21. After weaning, body weights of the dams were recorded weekly until scheduled sacrifice. Food consumption (g/rat/day) for both sexes was recorded weekly except during the mating period. In the females, food consumption was recorded daily during gestation and lactation. Relative food consumption (g/kg/day) and intake (mg/kg/day) were also calculated. Food efficiency (%) was also calculated for the appropriate intervals.

Vaginal lavages were performed daily and the slides evaluated to assess the regularity and duration of the estrous cycles of each P and F1 generation female for 21 days prior to pairing and continuing until mating was observed or until the end of the mating period. Additional lavages were performed on the day of necropsy to determine the stage of the estrous cycle.

2. **Litter observations:** The following litter parameters (X) were recorded (Table 2):

TABLE 2. F_1 / F_2 Litter observations [a]

Observation	Time of observation (post-natal day)						
	Day 0	Day 1	Day 4 (pre-cull)	Day 4 (post-cull)	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X	X	X
Number of dead pups	X	X	X	X	X	X	X
Pup weight		X	X	X	X	X	X
Sex of each pup (M/F)	X		X				X
External alterations	X						

a Data were obtained from pages 44 and 45 of the study report.

Litters were observed daily for mortality and clinical signs of toxicity and detailed physical examinations were performed on PND 1, 4, 7, 14, and 21. Wherever possible, any offspring found dead prior to PND 4 were examined externally and internally. On PND 4, litters were standardized to 8 pups each (4/sex where possible). Sexual maturation (vaginal patency or preputial separation) was evaluated. Males were evaluated for balanopreputial separation daily beginning on PND 35 and body weight was recorded on the day of attainment. Females were evaluated for vaginal patency daily beginning on PND 25 and body weight was recorded on the day of attainment.

1. **Postmortem observations**

a. **Parental animals:** All P generation adults were sacrificed by CO_2 inhalation following selection of the F1 generation and completion of a detailed clinical observation. All surviving F1 adults were similarly sacrificed following weaning of the F2 pups. All adults were subjected to a complete necropsy and selective histopathological examination. For females that failed to deliver, pregnancy status was determined, and specific emphasis was placed on anatomic or pathologic findings that may have interfered with pregnancy.

The following tissues from all P and F1 parents were collected (X) and weighed (XX). Organ-to-body weight ratios were calculated.

	FEMALE REPRODUCTIVE		BOTH SEXES		BOTH SEXES
XX	Ovaries	X	Salivary glands	X	Aorta
XX	Oviducts	X	Esophagus	X	Heart
XX	Uterus	X	Stomach	X	Bone marrow
XX	Cervix	X	Duodenum	X	Lymph nodes
X	Vagina	X	Jejunum	XX	Spleen
	MALE REPRODUCTIVE	X	Ileum	XX	Thymus
XX	Testes	X	Cecum	XX	Kidneys
XX	Epididymides	X	Colon	X	Urinary bladder
X	Vas deferens	X	Rectum	XX	Brain
XX	Prostate	XX	Liver	X	Peripheral nerve (sciatic)
XX	Seminal vesicles	X	Pancreas	X	Spinal cord (cervical)
XX	Coagulating glands	X	Trachea	XX	Pituitary
		X	Lung	X	Eyes (w/ optic nerve)
		XX	Adrenal gland	X	Bone (sternum)
		X	Parathyroid	X	Skeletal muscle
		XX	Thyroid	X	Skin w/ mammary gland
				X	All gross lesions and masses

a Data were obtained from pages 50 and 51 of the study report.

With the exception of the eyes with optic nerves (Davidson's solution) and testes and epididymides (Bouin's solution), the tissues listed above were fixed in 10% neutral buffered formalin. Tissues were processed routinely, embedded, sectioned at 2-4 microns, and stained with hematoxylin and eosin or PAS (right epididymis and testis). The following tissues from the P and F1 adults in the control and 1700 ppm groups and for all adults found dead or sacrificed *in extremis* were examined microscopically: adrenal glands, brain, cervix, coagulating gland, epididymis (right), kidneys, liver, ovaries, pituitary gland, prostate gland, seminal vesicles, spleen, testis (right), thymus gland, thyroid gland, uterus, vagina, vas deferens, and all gross lesions. In addition, the reproductive organs were examined for 300 and 600 ppm animals suspected of reduced fertility (failed to mate, conceive, sire, or deliver healthy offspring).

b. **Offspring:** All F1 offspring that were not selected for continuation of the study and all surviving F2 offspring were euthanized on PND 21 and subjected to gross necropsy. The brain, spleen, and thymus were collected and weighed from 1 pup/sex/litter and were fixed in 10% neutral buffered formalin for possible future examination.

D. DATA ANALYSIS

1. **Statistics:** Parental mating, fertility, conception and copulation indices were analyzed using the Chi-square test with Yates' correction factor. Mean parental (weekly, gestation and lactation) and offspring body weights and body weight changes, parental food consumption and food efficiency data, estrous cycle lengths, pre-coital intervals, gestation lengths, implantation sites, live litter sizes, unaccounted-for sites, numbers of pups born, balanopreputial separation data (day of attainment and body weight), vaginal patency data (day of attainment and body weight), absolute and relative organ weights, sperm production rates, epididymal and testicular sperm numbers, and ovarian primordial follicle counts were

subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant intergroup variance, Dunnett's test was used to compare the test article-exposed groups to the control group. Mean litter proportions (percent per litter) of postnatal pup survival and pup sexes at birth (percentage of males per litter), percentages of motile sperm, and percentages of sperm with normal morphology were subjected to the Kruskal-Wallis nonparametric ANOVA test to determine intergroup differences. If the ANOVA revealed statistically significant intergroup variance, Dunn's test was used to compare the test article-exposed groups to the control group. Histopathological findings in the test article-exposed groups were compared to the control group using a two-tailed Fisher's Exact test.

Data from non-gravid animals were excluded from statistical analysis following the mating period. Statistical significance was denoted at $p<0.05$ and $p<0.01$. The reviewers consider the statistical methods used to be appropriate.

2. **Indices**

Reproductive indices: The following reproductive indices were calculated by the performing laboratory from breeding and parturition records of animals in the study:

Mating index (%) = No. of animals with evidence of mating/No. of animals paired x 100

Male fertility index (%) = No. of males siring a litter/ No. of males used for mating x 100

Male copulation index (%) = No. of males siring a litter/ No. of males with evidence of mating x 100

Fertility index (%) = No. of females pregnant/ No. of females paired x 100

Conception index (%) = No. of females pregnant/ No. of females with evidence of mating x 100

The following index was calculated by the reviewers from the litter data:

Gestation index (%) = No. of live litters born/ No. of pregnant females x 100

Offspring viability indices: The following offspring indices were calculated by the reviewers:

Live birth index (%) = No. of live pups on PND 0/total No. of pups on PND 0 x 100

Viability index (%) = No. of live pups on PND 4 (pre-cull)/ No. of live pups on PND 0 x 100

Lactation index (%) = No. of live pups on PND 21/ No. of live pups on PND 4 (post-cull) x

100

3. **Historical control data:** Historical control data for reproductive parameters were provided on pages 3345-3353 of the study report.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs

a. **Mortality:** All P generation parents survived to scheduled sacrifice. The following F1 adults were found dead or sacrificed during the study: one 300 ppm male (No. 19265-05) was sacrificed *in extremis* during Week 27 (prior to mating) as a result of a fractured frontal bone; one 600 ppm male (No. 19304-02) was found dead during Week 32, there were no clinical signs observed prior to death and the cause of death was undetermined; and one 1700 ppm female (No. 19280-17) was found dead during Week 23 (prior to mating) as a result of chronic biliary hepatitis, which was considered to be unrelated to treatment. All other F1 adults survived to scheduled sacrifice.

b. **Clinical signs of toxicity:** There were no remarkable clinical observations in the P or F1 generation males or females.

2. Body weight, body weight gain, and food consumption

a. **Pre-mating:** Pre-mating body weights and overall body weight gains for the P and F1 generations are presented in Table 3a below.

In the P generation, body weight gains were decreased ($p<0.01$) at 1700 ppm in the males (↓18%) and females (↓29%) during week 0-1 of the pre-mating period. Overall pre-mating (Weeks 0-10) body weight gain was decreased ($p<0.05$) by 10% in the 1700 ppm females, but was not significantly different from controls in the males. Slight decreases in body weight (↓4%, $p<0.05$ for wk 1) and (↓5%, $p<0.01$ for wk 3) were noted in the 1700 ppm males. No treatment-related effects on body weights or overall body weight gains were noted at 300 and 600 ppm in either sex.

In the F1 generation, body weights were decreased at 1700 ppm throughout the pre-mating period (weeks 18-28) by 6-17% in the males ($p<0.05$) and 7-17% in the females ($p<0.01$). Body weight gains were decreased ($p<0.05$) by 8% in the 1700 ppm males during week 18-19. Overall pre-mating (weeks 18-28) body weight gains were similar to controls at all doses in both sexes.

Pre-mating absolute (g/rat/day) and relative (g/kg/day) food consumption and food efficiency (%) for the P and F1 generations are presented in Table 3b below. In the 1700 ppm P

generation males, absolute food consumption was decreased ($p<0.01$) by 4-11% during weeks 0-1, 2-3, and 3-4. During week 0-1, relative food consumption was decreased ($p<0.01$) by 9% in this group and food efficiency was decreased ($p<0.01$) by 10% relative to controls. In the 1700 ppm P generation females, absolute food consumption was decreased by 5% each during weeks 0-1 ($p<0.01$) and 1-2 ($p<0.05$), and relative food consumption was decreased ($p<0.01$) by 7% during week 0-1. Food efficiency was similar to controls at all doses.

In the 1700 ppm F1 generation males, absolute food consumption was decreased ($p<0.05$) by 3-13% during weeks 18-19 ($p<0.01$), 19-20, 20-21, and 22-23, and relative food consumption and food efficiency were similar to controls in this group. In the 1700 ppm F1 generation females, absolute food consumption was decreased ($p<0.01$) by 8-11% during Weeks 18-19 through 25-26. Relative food consumption in this group was similar to controls throughout pre-mating. Food efficiency was increased ($p<0.05$) by 8% relative to controls during Week 18-19.

All differences in body weight, body weight gain, relative and absolute food consumption and/or food efficiency noted at 300 and 600 ppm in the P and F1 generations were considered unrelated to treatment because they were minor, sporadic, and/or unrelated to dose.

TABLE 3a. Mean (±SD) body weight and body weight gain (g) during pre-mating. [a]

Parameter	Weeks	Dietary concentration (ppm)			
		0	300	600	1700
P generation males					
Body weight	0	225±11.2	224±10.7	224±10.2	224±11.3
	1	274±16.0	268±11.6	274±14.1	264±16.3* (↓4%)
	3	362 ± 24.7	351 ± 22.1	356 ± 21.7	344±24.7** (↓5%)
	10	508 ± 47.1	497 ± 38.9	502 ± 36.0	489±43.3
Body weight gain	0-1	49±8.3	44±6.4* (↓10%)	49±6.8	40±9.2** (↓18%)
	0-10	284±43.8	274±34.3	278±29.9	265±34.9
P generation females					
Body weight	0	172 ± 9.1	172 ± 10.7	172 ± 10.7	173 ± 10.7
	10	298 ± 18.7	289 ± 25.2	296 ± 23.6	287 ± 19.9
Body weight gain	0-1	21±9.1	17±5.6	18±6.0	15±7.3** (↓29%)
	0-10	126±14.2	117±18.8	124±17.7	114±14.4* (↓10%)
F1 generation males					
Body weight	18	128 ± 15.5	121 ± 14.9	124 ± 15.0	106±18.3** (↓17%)
	23	403 ± 49.7	401 ± 33.8	399 ± 39.5	374±33.8* (↓7%)
	28	532 ± 50.7	527 ± 48.7	530 ± 57.5	498±52.3* (↓6%)
Body weight gain	18-19	64 ± 7.4	63 ± 6.1	61 ± 7.1	59±7.7* (↓8%)
	18-28	409 ± 37.7	406 ± 46.2	407 ± 47.0	393 ± 47.6
F1 generation females					
Body weight	18	112 ± 10.3	108 ± 10.9	107 ± 11.5	93±14.1** (↓17%)
	23	252 ± 25.8	251 ± 23.8	245 ± 21.7	233±18.7** (↓8%)
	28	305 ± 26.0	300 ± 28.9	297 ± 26.2	283±22.1** (↓7%)
Body weight gain	18-28	197 ± 20.6	192 ± 24.7	190 ± 23.0	190 ± 19.6

a Data were obtained from Tables 3-7 and 55-60 on pages 107-125 and 247-266 of the study report; n=30. Percent difference from controls is presented parenthetically.

* Significantly different from controls at $p<0.05$

** Significantly different from controls at $p<0.01$

TABLE 3b. Mean (±SD) absolute (g/rat/day) and relative (g/kg/day) food consumption (FC) and food efficiency (%) during pre-mating. [a]

Parameter	Weeks	Dietary concentration (ppm)			
		0	300	600	1700
P generation males					
Absolute FC	0-1	28±2.7	26±2.3* (↓7%)	28±2.2	25±2.4** (↓11%)
	2-3	28 ± 2.3	27 ± 2.0	27 ± 2.6	27±2.1* (↓4%)
	3-4	29 ± 2.9	28 ± 2.0	27 ± 2.5	27±2.1** (↓7%)
	9-10	30 ± 3.2	29 ± 2.6	29 ± 2.3	29 ± 2.3
Relative FC	0-1	112 ± 9.5	106±9.5* (↓5%)	111 ± 7.6	102±8.5** (↓9%)
	9-10	59 ± 3.8	59 ± 3.8	59 ± 2.8	60 ± 2.8
Food Efficiency	0-1	25.1 ± 3.1	24.3 ± 3.7	25.3 ± 2.5	22.6 ± 4.7** (↓10%)
	9-10	8.0 ± 2.2	7.5 ± 2.9	8.5 ± 2.0	8.2 ± 1.6
P generation females					
Absolute FC	0-1	20 ± 2.1	19 ± 1.5	20 ± 1.7	19±2.2** (↓5%)
	1-2	20 ± 2.1	20 ± 1.9	20 ± 1.9	19±3.0* (↓5%)
	9-10	22 ± 1.3	21 ± 1.9	21 ± 2.0	21 ± 2.3
Relative FC	0-1	112 ± 12.6	107 ± 8.0	111 ± 6.1	104±14.2** (↓7%)
	9-10	74 ± 5.8	73 ± 4.5	73 ± 4.6	73 ± 6.6
Food Efficiency	0-1	14.8 ± 6.6	12.4 ± 3.8	12.5 ± 4.1	11.4 ± 5.9
	9-10	6.2 ± 3.2	5.5 ± 3.2	6.3 ± 2.9	5.1 ± 2.7
F1 generation males					
Absolute FC	18-19	23 ± 2.0	23 ± 1.8	23 ± 2.4	20±2.6** (↓13%)
	20-21	29 ± 3.7	30 ± 2.1	29 ±2.2	28±2.6* (↓3%)
	22-23	32 ± 3.2	32 ± 2.6	31 ± 2.5	30±2.4* (↓6%)
	27-28	32 ± 2.8	31 ± 2.6	31 ± 2.8	30 ± 4.1
Relative FC	18-19	145 ± 10.1	149 ± 10.5	148 ± 8.3	152 ± 12.7
	27-28	61 ± 3.9	59 ± 3.0	60 ± 2.6	60 ± 5.2
Food Efficiency	18-19	39.8 ± 3.5	39.8 ± 3.2	38.5 ± 2.4	41.6 ± 5.5
	27-28	9.2 ± 2.5	9.4 ±2.2	10.1 ± 3.0	8.1 ± 3.4
F1 generation females					
Absolute FC	18-19	19 ± 1.4	19 ± 1.6	19 ± 1.9	17±1.8** (↓11%)
	20-21	22 ± 1.9	22 ± 2.1	21±2.0* (↓5%)	20±1.6** (↓9%)
	23-24	24 ± 2.4	23 ± 2.3	23 ± 2.6	22±1.6** (↓8%)
	27-28	22 ± 1.5	21 ± 2.2	21 ± 2.3	21 ± 2.0
Relative FC	18-19	146 ± 11.7	148 ± 10.4	152 ± 12.2	154 ± 11.8
	27-28	73 ± 5.2	72 ± 7.6	72 ± 4.9	74 ± 5.4
Food Efficiency	18-19	31.2 ± 3.4	30.9 ± 3.0	30.4 ± 4.0	33.6±3.5* (↑8%)
	27-28	4.6 ± 3.0	4.7 ± 2.9	4.3 ± 3.7	4.2 ± 4.7

a Data were obtained from Tables 13-18 and 65-70 on pages 133-156 and 273-296 of the study report; n=30. Percent difference from controls is presented parenthetically.

* Significantly different from controls at $p<0.05$

** Significantly different from controls at $p<0.01$

b. Gestation: Body weights, body weight gains, absolute and relative food consumption, and food efficiency data for the P and F1 generation females during gestation are presented in Table 4 below.

Mean P generation maternal body weights, body weight gains, absolute (g/animal/day) and relative (g/kg/day) food consumption, and food efficiency (%) were unaffected by treatment during gestation.

Mean F1 generation maternal body weights were decreased by up to 6% at 1700 ppm compared to controls during gestation. However, these decreases did not attain statistical significance and there were no significant decreases in body weight gains during gestation. These decreases in body weight were considered to be a continuation of the lower mean body weights noted for these females during pre-mating. Overall (GD 0-20) body weight gains were similar to controls at all doses. Mean F1 maternal absolute and relative food consumption, and food efficiency were unaffected by treatment during gestation.

All differences in body weight, body weight gain, relative or absolute food consumption and/or food efficiency noted at 300 and 600 ppm in the P and F1 generations during gestation were considered unrelated to treatment because they were minor, sporadic, and/or unrelated to dose.

TABLE 4. Mean (±SD) body weights, body weight gains (g), absolute (g/rat/day) and relative (g/kg/day) food consumption (FC) during gestation in P and F1 generation females. [a]

Parameter	GD	Dietary concentration (ppm)			
		0	300	600	1700
P generation					
Body weight	0	293 ± 19.4	286 ± 23.4	291 ± 22.1	282 ± 18.7
	20	421 ± 24.2	418 ± 30.0	418 ± 32.6	409 ± 23.2
Body weight gain	0-20	128 ± 21.5	131 ± 15.0	127 ± 18.2	126 ± 21.0
Absolute FC	0-20	24 ± 1.9	23 ± 2.4	23 ± 1.9	23 ± 1.7
Relative FC	0-20	69 ± 5.1	67 ± 6.7	68 ± 4.5	68 ± 3.7
Food efficiency	0-20	27.0 ± 4.5	29.5 ± 5.2	27.4 ± 2.8	28.0 ± 4.3
F1 generation					
Body weight	0	298 ± 26.8	298 ± 29.4	294 ± 26.6	283 ± 23.2
	20	431 ± 33.1	426 ± 38.5	426 ± 34.6	409 ± 40.4
Body weight gain	0-20	132 ± 18.5	128 ± 18.1	132 ± 18.5	126 ± 28.2
Absolute FC	0-20	24 ± 1.7	24 ± 3.2	24 ± 2.0	23 ± 2.2
Relative FC	0-20	70 ± 4.5	68 ± 7.6	69 ± 4.5	70 ± 4.5
Food efficiency	0-20	27.3 ± 3.6	27.0 ± 3.9	27.4 ± 3.3	26.9 ± 5.7

a Data were obtained from Tables 9, 10, 19-21, 61, 62, and 71-73 on pages 127-130, 157-162, 267-270, and 297-302 of the study report. Percent difference from controls is presented parenthetically.

c. **Lactation:** Body weights, body weight gains, absolute and relative food consumption, and food efficiency data for the P and F1 generation females during lactation are presented in Table 5 below.

In the 1700 ppm P generation females, maternal body weights were decreased ($p<0.05$) by 5% each on LD 4 and 14. Body weight gain was decreased ($p<0.05$) by 64% during LD 1-4 in this group and increased by 83% during LD 4-7. Overall (LD 1-21) body weight gain was increased ($p<0.01$) by 59% compared to controls. Absolute and relative food consumption were decreased by 15 ($p<0.01$) and 12% ($p<0.05$), respectively, during LD 1-4 in this group. Overall (LD 1-21) food efficiency was increased by 58% relative to controls at this dose.

In the 1700 ppm F1 females, maternal body weights were decreased by 6% ($p<0.05$) on LD 4. Overall (LD 1-21) body weight gains were similar to controls at all doses. Mean F1 maternal absolute and relative food consumption and food efficiency were unaffected by treatment during lactation.

All differences in body weight, body weight gain, relative or absolute food consumption and/or food efficiency noted at 300 and 600 ppm in the P and F1 generations during lactation were considered unrelated to treatment because they were minor, sporadic, and/or unrelated to dose.

TABLE 5. Mean (±SD) body weights, body weight gains (g), absolute (g/rat/day) and relative (g/kg/day) food consumption (FC) during lactation in P and F1 generation females. [a]

Parameter	LD	Dietary concentration (ppm)			
		0	300	600	1700
P generation					
Body weight	1	322 ± 18.4	320 ± 24.1	320 ± 25.7	311 ± 20.7
	4	333 ± 21.2	324 ± 23.2	328 ± 26.4	315±16.7* (↓5%)
	14	363 ± 18.4	361 ± 25.0	358 ± 25.1	346±17.7* (↓5%)
	21	350 ± 20.7	354 ± 22.9	357 ± 25.0	357 ± 19.0
Body weight gain	1-4	11 ± 10.2	4±9.6* (↓64%)	8 ± 9.3	4±10.7* (↓64%)
	4-7	6 ± 7.6	12±7.7** (↑100%)	6 ± 9.2	11±6.9* (↑83%)
	1-21	29 ±15.7	37 ± 15.4	37 ± 21.4	46±12.9** (↑59%)
Absolute FC	1-4	39 ± 7.3	34 ± 5.9 *(↓13%)	37 ± 4.6	33±6.0** (↓15%)
	1-21	56 ± 5.2	54 ± 8.9	57 ± 5.5	55 ± 4.7
Relative FC	1-4	119 ± 22.2	106±19.9* (↓11%)	113 ± 13.7	105±19.4* (↓12%)
	1-21	166 ± 15.7	164 ± 21.1	168 ± 15.0	167 ± 13.8
Food efficiency	4-7	4.4 ± 6.48	9.9±6.36** (↑125%)	4.7 ± 7.46	9.0±5.96* (↑105%)
	1-21	2.6 ± 1.39	3.3 ± 1.39	3.3 ± 2.01	4.1±1.16* (↑58%)
F1 generation					
Body weight	1	329 ± 26.5	329 ± 32.0	330 ± 28.4	314 ± 23.6
	4	342 ± 27.5	343 ± 28.8	343 ± 27.1	322±26.4* (↓6%)
	21	356 ± 22.2	355 ± 27.3	353 ± 27.6	347 ± 25.4
Body weight gain	1-21	27 ± 15.8	27 ± 17.3	21 ± 15.7	34 ± 13.9
Absolute FC	1-21	57 ± 4.9	56 ± 6.8	57 ± 9.3	56 ± 4.4
Relative FC	1-21	165 ± 12.8	162 ± 19.5	168 ± 11.7	169 ± 9.4
Food efficiency	1-21	2.4 ± 1.33	2.4 ± 1.54	1.8 ± 1.38	3.0 ± 1.17

a Data were obtained from Tables 11, 12, 22-24, 63, 64, and 74-76 on pages 131-132, 163-165, 271-272, and 303-305 of the study report. Percent difference from controls is presented parenthetically.

* Significantly different from controls at $p<0.05$

** Significantly different from controls at $p<0.01$

3. **Test substance intake:** The mean test substance intake for both generations during pre-mating (calculated by reviewers) is considered to be representative of the achieved intake for the entire study (Table 6).

TABLE 6. Mean test substance intake (mg/kg/day in males/females) during pre-mating. [a]				
Generation	Dietary concentration (ppm)			
	0	300	600	1700
P generation	0/0	22/25	45/52	127/144
F1 generation	0/0	27/29	53/59	155/169
Mean [b]	**0/0**	**25/27**	**49/56**	**141/157**

a Values were calculated by reviewers using data obtained from pages 62 and 76 of the study report.
b Calculated by the reviewers as the average of the P and F1 generations separately for each sex.

4. Reproductive function

a. Estrous cycle length: Mean estrous cycle lengths were unaffected by treatment in both the P and F1 generation.

b. Spermatogenic endpoints: No treatment-related effects were observed on P or F1 generation spermatogenesis endpoints (mean testicular and epididymal sperm numbers, sperm production rate, motility, progressive motility, and morphology).

5. Reproductive performance: No treatment-related effects on the mating, fertility, conception/copulation, or gestation indices were observed in either generation (Table 7). There were no effects of treatment on pre-coital interval or gestation duration in either generation. No signs of dystocia were noted at any dose.

TABLE 7. Reproductive performance [a]					
Parameter		Dietary concentration (ppm)			
		0	300	600	1700
P generation					
Mean pre-coital interval (days)		2.8	2.6	2.5	2.3
Number paired	Males	30	30	30	30
	Females	30	30	30	30
Number of females with evidence of mating		29	29	30	28
Copulation index (%, ♂)		100	93.1	96.7	92.9
Conception index (%, ♀)		100	93.1	96.7	93.1
Mating index (%)	Males	96.7	96.7	100	96.7
	Females	96.7	96.7	100	96.7
Number pregnant		29	27	29	27
Fertility index (%)	Males	96.7	90.0	96.7	90.0
	Females	96.7	90.0	96.7	90.0
Gestation index (%)		96.6	96.3	100	96.3
Gestation duration (mean # days)		21.8	21.9	21.8	21.9
F1 generation					
Mean pre-coital interval (days)		2.7	2.8	2.4	2.5
Number paired	Males	30	30	30	30
	Females	30	30	30	29
Number of females with evidence of mating		28	28	30	28
Copulation index (%, ♂)		89.3	96.3	90.0	96.4
Conception index (%, ♀)		89.3	96.4	90.0	96.4
Mating index (%)	Males	93.3	93.1	100	96.6
	Females	93.3	93.3	100	96.6
Number pregnant		25	27	27	27
Fertility index (%)	Males	83.3	89.7	90.0	93.1
	Females	83.3	90.0	90.0	93.1
Gestation index (%)		100	96.3	96.3	96.3
Gestation duration (mean # days)		21.6	21.6	21.8	21.9

a Data were obtained from Tables 29, 31, 81, and 83 on pages 177, 179, 181, 317, 319, and 321 of the study report.

6. Parental postmortem results

a. Organ weights: No treatment-related effects on P or F1 generation absolute or relative (to body) organ weights were observed at any dose in either sex.

In the F1 generation, statistically significant ($p<0.05$) increases in relative (to body) organ weights were noted at 1700 ppm in the males (kidney and testes) and females (kidney). However, as these increases occurred in the absence of increased absolute organ weights, and there were no corroborative histopathological findings in either of these tissues during microscopic examination, they were attributed to the lower terminal body weights in these animals, and were considered to be unrelated to treatment.

All other statistically significant findings were considered unrelated to treatment because they were minor, not corroborated with histopathological findings, and/or were unrelated to dose.

b. Pathology

1) **Macroscopic examination:** There were no treatment-related gross findings in the P or F1 males or females at any dose.

2) **Microscopic examination:** No treatment-related microscopic findings were observed at any dose in either sex in either generation. The statistically significant ($p<0.05$) decrease in the incidence of implantation sites in the uterus of the 1700 ppm P generation females was considered to be incidental and most likely associated with the unavoidable differences in the plane of sectioning of the tissue. As there were no decreases in the mean number of implantation sites as observed grossly in the uterus or in the number of pups born for these females, this finding was considered to be unrelated to treatment.

B. OFFSPRING

1. **Viability and clinical signs:** Litter data for the F1 and F2 litters are included in Table 8. No adverse clinical signs were observed in the F1 or F2 generation pups from birth to PND 21 at any dose. No treatment-related differences in litter parameters were noted at any dose in either generation.

TABLE 8. Litter Parameters [a]

Parameter	Dietary concentration (ppm)			
	0	300	600	1700
F1 litter				
Number of litters	28	27	29	26
Mean (±SD) implantation sites	16.0±2.4	15.3±2.2	15.8±2.0	15.3±2.4
Number born live	420	397	424	372
Number stillborn	3	1	13	9
Sex ratio on PND 0 (%♂)	46.7±12.7	53.3±13.1	48.9±12.4	54.4±14.9
Mean litter size, PND 0	15.0±2.3	14.7±2.4	14.6±2.0	14.3±2.8
PND 4 (pre-cull)[b]	14.68	13.63	14.31	14.00
PND 4 (post-cull)[b]	7.93	7.48	7.97	7.92
PND 7[b]	7.82	7.48	7.90	7.88
PND 14[b]	7.82	7.44	7.83	7.85
PND 21[b]	7.75	7.44	7.83	7.85
Live birth index (%)[b]	99.3	99.7	97.0	97.6
Number of females with total litter death	0	1	0	0
Viability index (%)[b]	97.9	92.7	97.9	97.8
Lactation index (%)[b]	97.7	99.5	98.3	99.0
F2 litter				
Number of litters	25	26	27	26
Mean (±SD) implantation sites	15.4±2.1	14.7±1.6	14.5±2.6	15.1±2.8
Number born live	364	360	372	365
Number stillborn	3	3	5	7
Sex ratio on PND 0 (%♂)	48.8±17.9	53.8±11.8	52.3±11.9	49.2±12.1
Mean litter size, PND 0	14.6±2.4	13.8±1.7	13.8±2.7	14.1±2.6
PND 4 (pre-cull)[b]	14.28	13.42	13.07	13.27
PND 4 (post-cull)[b]	7.96	8.00	7.70	8.04
PND 7[b]	7.88	7.96	7.67	8.04
PND 14[b]	7.84	7.92	7.63	8.00
PND 21[b]	7.84	7.81	7.63	8.00
Live birth index (%)[b]	99.2	99.2	98.7	98.1
Number of females with total litter death	0	0	1	0
Viability index (%)[b]	98.1	96.9	94.9	94.5
Lactation index (%)[b]	98.5	97.6	99.0	99.5

a Data were obtained from Tables 29, 37, 42, 81, 91, and 99 on pages 177, 193, 223, 317, 336, and 375 of the study report.

b Calculated by reviewers from data from Tables A42 and A98 on pages 1266-1272 and 2629-2633 of the study report.

2. **Body weight:** Pup body weight data are presented in Table 9. At 1700 ppm, overall (PND 1-21) pup body weight gains (calculated by reviewers) were decreased by 10-13% in the males and 9-12% in the females of both generations. Body weights at this dose were decreased ($p<0.05$) by 10-11% in the F1 generation and by 7-8% in the F2 generation on PND 21.

Table 9. Mean (±SD) body weights and body weight gains (g) in F1 and F2 generation pups. [a]					
Parameter		Dietary concentration (ppm)			
		0	300	600	1700
F1 males					
Body weight	PND 1	6.8 ± 0.67	7.1 ± 0.53	7.0 ± 0.62	7.0 ± 0.62
	PND 4	9.2±1.18	9.7±0.96	9.7±1.05	9.9±1.31
	PND 7	14.8 ± 2.60	14.6 ± 2.74	14.9 ± 2.48	14.6 ± 2.40
	PND 14	31.2 ±5.72	30.8 ±4.13	30.0 ±4.52	29.4 ± 3.12
	PND 21	50.4 ± 9.30	49.2 ± 5.69	48.0 ± 7.93	45.0±3.83* (↓11%)
Body weight gain[b]	PND 1-21	43.6	42.1	41.0	38.0 (↓13%)
F1 females					
Body weight	PND 1	6.4 ± 0.54	6.8 ± 0.52	6.5 ± 0.55	6.6 ± 0.73
	PND 4	8.7 ± 1.05	9.3 ± 0.89	9.2 ±0.89	9.2 ±1.36
	PND 7	14.1 ± 2.52	13.8 ± 2.31	13.8 ± 2.32	13.8 ± 2.10
	PND 14	30.0 ± 5.61	29.5 ± 4.16	28.9 ± 4.61	28.1 ± 2.71
	PND 21	47.7 ± 8.84	47.0 ±5.38	45.8 ± 7.55	42.9±3.71 (↓10%)
Body weight gain[b]	PND 1-21	41.3	40.2	39.3	36.3 (↓12%)
F2 males					
Body weight	PND 1	6.9 ± 0.74	7.0 ± 0.71	7.0 ± 0.72	7.1 ± 0.64
	PND 4	10.1 ± 1.42	10.1 ± 1.53	10.6 ± 1.17	10.2 ± 1.22
	PND 7	16.1 ± 2.34	16.0 ± 2.75	16.9 ± 1.68	15.8 ± 1.80
	PND 14	33.2 ± 3.25	32.9 ± 5.11	34.6 ± 2.52	32.1 ± 3.25
	PND 21	51.4 ± 4.85	48.5 ± 8.78	53.4 ± 3.99	47.3±5.07* (↓8%)
Body weight gain[b]	PND 1-22	44.5	41.5	46.4	40.2 (↓10%)
F2 females					
Body weight	PND 1	6.5 ± 0.70	6.6 ±0.63	6.6 ± 0.77	6.8 ± 0.56
	PND 4	9.5 ± 1.45	9.5 ± 1.48	10.1 ± 1.27	9.6 ± 1.07
	PND 7	15.2 ± 2.18	15.1 ± 2.56	15.8 ± 2.07	14.9 ± 1.74
	PND 14	31.9 ± 3.42	31.4 ± 4.82	32.9 ± 2.90	31.1 ± 2.86
	PND 21	48.8 ± 3.90	46.4 ± 7.35	50.5 ± 4.37	45.2±4.26* (↓7%)
Body weight gain[b]	PND 1-21	42.3	39.8	43.9	38.4 (↓9%)

a Data were obtained from Tables 45 and 102 on pages 227-229 and 379-381 of the study report. Percent difference from controls is presented parenthetically.
b Calculated by reviewers using data within this table.
* Significantly different from controls at $p<0.05$

3. **Sexual maturation:** No treatment-related effects on age of attainment of vaginal patency or body weight at the age of attainment were noted at any dose in the F1 females.

   The slightly higher value for age of attainment of balanopreputial separation noted in the F1 males at 1700 ppm (47.1 days treated vs. 45.0 days controls) was within the range of historical controls (42.0-49.0 days) and was attributed to the lower mean body weights of these animals (226.6 g) compared to controls (237.1 g).

4. **Offspring postmortem results**

a) **Organ weights:** No treatment-related effects on organ weights were observed in the F1 generation pups.

In the F2 generation pups, treatment-related decreases ($p<0.05$) in absolute and relative (to body) spleen weights were observed in both sexes (↓9-19%) on PND 21 (Table 10). No other treatment-related effects on organ weights were observed in this generation. The increases in relative brain weight noted at 1700 ppm in both sexes were considered to be a result of the decreased terminal body weights in both sexes at this dose.

TABLE 10. Selected absolute (g) and relative (to body, %) organ weights in the F2 generation. [a]

Observation	Dose (ppm)			
	0	300	600	1700
Males				
Terminal BW (g)	51 ± 5.1	48 ± 9.9	54 ± 4.9	46±8.7* (↓10)
Absolute Spleen	0.217 ± 0.039	0.202 ± 0.057	0.218 ± 0.038	0.176±0.038** (↓19)
Relative Spleen	0.420 ± 0.054	0.417 ± 0.065	0.404 ± 0.056	0.382±0.048 (↓9)
Absolute Brain	1.46 ± 0.064	1.44 ± 0.139	1.48 ± 0.067	1.46 ± 0.135
Relative Brain	2.86 ± 0.237	3.08 ± 0.506	2.78 ± 0.245	3.26 ± 0.660**(↑14)
Females				
Terminal BW (g)	50 ± 4.5	47 ± 8.0	51 ± 4.1	46±5.3* (↓8)
Absolute Spleen	0.217 ± 0.038	0.204 ± 0.055	0.218 ± 0.039	0.175±0.034** (↓19)
Relative Spleen	0.435 ± 0.058	0.427 ± 0.069	0.425 ± 0.060	0.381±0.055** (↓12)
Absolute Brain	1.40 ± 0.069	1.41 ± 0.102	1.45 ± 0.056	1.42 ± 0.062
Relative Brain	2.83 ± 0.265	3.04 ± 0.394* (↑7)	2.86 ± 0.222	3.14 ± 0.306**(↑11)

a Data were obtained from Table 107 on pages 388-391 of the study report. Percent difference from controls is presented parenthetically.

* Significantly different from controls at $p<0.05$

** Significantly different from controls at $p<0.01$

b) Pathology

1) **Macroscopic examination:** No macroscopic findings could be attributed to treatment in the F1 or F2 pups.

2) **Microscopic examination:** No microscopic findings could be attributed to treatment in the F1 pups.

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that the parental LOAEL was 1700 ppm based on decreases in body weight and food consumption in both sexes in both generations. The offspring LOAEL was 1700 ppm based on decreased body weights and body weight gains near the end of the lactation period, at a time when pups began direct consumption of test diets. In addition, lower spleen weights were observed in the F2 pups. There were no functional effects on reproductive parameters at any dose tested; therefore, the reproductive toxicity NOAEL was 1700 ppm.

B. REVIEWER COMMENTS

1. **PARENTAL ANIMALS:** All P generation parents survived to scheduled sacrifice. Three F1 adults were found dead or sacrificed during the study; however, none of these mortalities were related to treatment. All other F1 adults survived to scheduled sacrifice.

   In the P generation, body weight gains were decreased at 1700 ppm in both sexes during Week 0-1 of the pre-mating period. Overall pre-mating (Weeks 0-10) body weight gain was decreased in the 1700 ppm females, but was not significantly different from controls in the males. Slight decreases in body weight were noted in the 1700 ppm males during Weeks 1-3. In the 1700 ppm P generation males, absolute food consumption was decreased during Weeks 0-1, 2-3, and 3-4. During Week 0-1, relative food consumption was decreased in this group and food efficiency was decreased relative to controls. In the 1700 ppm P generation females, absolute food consumption was decreased during Weeks 0-1 and 1-2, and relative food consumption was decreased during Week 0-1. Food efficiency was similar to controls at all doses. Mean P generation maternal body weights, body weight gains, absolute and relative food consumption, and food efficiency were unaffected by treatment during gestation. In the 1700 ppm P generation females, maternal body weights were decreased on LD 4 and 14. Body weight gain was decreased during LD 1-4 in this group and increased during LD 4-7. Overall (LD 1-21) body weight gain was increased compared to controls. Absolute and relative food consumption was decreased during LD 1-4 in this group. Overall (LD 1-21) food efficiency was increased relative to controls at this dose.

   In the F1 generation, body weights were decreased at 1700 ppm throughout the pre-mating period (Weeks 18-28) in both sexes. Body weight gains were decreased in the males at this dose during Week 18-19. Overall pre-mating (Weeks 18-28) body weight gains were similar to controls at all doses in both sexes. In the 1700 ppm F1 generation males, absolute food consumption was decreased during Weeks 18-19, 19-20, 20-21, and 22-23, and relative food consumption and food efficiency were similar to controls in this group. In the 1700 ppm F1 generation females, absolute food consumption was decreased during Weeks 18-19 through 25-26. Relative food consumption in this group was similar to controls throughout pre-mating. Food efficiency was increased relative to controls during Week 18-19. Mean F1 generation maternal body weights were decreased at 1700 ppm compared to controls during gestation. However, these decreases did not attain statistical significance and there were no significant decreases in body weight gains during gestation. These decreases in body weight were considered to be a continuation of the lower mean body weights noted for these females during pre-mating. Overall (GD 0-20) body weight gains were similar to controls at all doses. Mean F1 maternal absolute and relative food consumption, and food efficiency were unaffected by treatment during gestation. In the 1700 ppm F1 females, maternal body weights were decreased on LD 4. Overall (LD 1-21) body weight gains were similar to controls at all doses. Mean F1 maternal absolute and relative food consumption, and food efficiency were unaffected by treatment during lactation.

   No adverse effects of treatment were noted at 300 or 600 ppm in either generation.

The LOAEL for parental toxicity is 1700 ppm (equivalent to 141/157 mg/kg/day in males/females, respectively) based on decreased body weights, body weight gains, and food consumption. The NOAEL is 600 ppm (equivalent to 49/56 mg/kg/day in males/females, respectively).

2. **OFFSPRING:** No adverse clinical signs were observed in the F1 or F2 generation pups from birth to PND 21 at any dose. No treatment-related differences in litter parameters were noted at any dose in either generation. Sexual maturation was unaffected by treatment. There were no treatment-related effects on gross pathology or histopathology.

   At 1700 ppm, overall (PND 1-21) pup body weight gains were decreased in the males and females of both generations. Body weights at this dose were decreased in the F1 and F2 generations on PND 21.

   In the F2 generation pups, treatment-related decreases in absolute and relative spleen weights were observed in both sexes on PND 21.

   The LOAEL for offspring toxicity is 1700 ppm (equivalent to 141/157 mg/kg/day in males/females, respectively) based on decreased body weights, body weight gains, and spleen weight (F2 pups only). The NOAEL is 600 ppm (equivalent to 49/56 mg/kg/day in males/females, respectively).

3. **REPRODUCTIVE TOXICITY:** No treatment-related effects on the mating, fertility, conception/copulation, or gestation indices were observed in either generation. There were no effects of treatment on pre-coital interval or gestation duration in either generation. No signs of dystocia were noted at any dose. Mean estrous cycle lengths were unaffected by treatment in both generations. No treatment-related effects were observed on P or F1 generation spermatogenesis endpoints (mean testicular and epididymal sperm numbers, sperm production rate, motility, progressive motility, and morphology).

   The LOAEL for reproductive toxicity was not observed. The NOAEL is 1700 ppm (equivalent to 141/157 mg/kg/day in males/females, respectively).

   This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3800; OECD 416) for a two-generation reproduction study in the rat.

IV. **STUDY DEFICIENCIES:** No deficiencies were noted.

EPA Reviewer: Jenny J. Tao **Signature:**
Risk Assessment and Science Support Branch,
Antimicrobial Division **Date** 6/21/11

EPA Secondary Reviewer: Jonathan Chen, Ph.D. **Signature:**
Risk Assessment and Science Support Branch,
Antimicrobial Division **Date** 06/21/2011

TXR #: 1,003,229

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity in Dogs (feeding); OPPTS 870.3150 ['82-1b]; OECD 409.

PC CODE: 098951 **DP BARCODE:** D384564

TEST MATERIAL (PURITY): N-butyl-1,2-benzisothiazolin-3-one (99.4% a.i.)

SYNONYMS: 2-Butyl-1,2-benzisothiazolin-3(2H)-one; BBIT

CITATION: Mertens, J. (2007) A 90-day oral (capsule) toxicity study of N-Butyl Benzisothiazolin-3-One in beagle dogs. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Study No.: WIL-544002, 19 February 2007. MRID 48262204. Unpublished.

SPONSOR: Arch Chemicals, Inc., 501 Merritt 7, P.O. Box 5204, Norwalk, CT

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 48262204), N-butyl-1,2-benzisothiazolin-3-one (99.4% a.i.; Lot No. 6180) in the vehicle, corn oil, was administered by gelatin capsule, once daily, to four beagle dogs/sex/dose group at doses of 0, 25, 75 or 250 mg/kg/day for at least 90 days. The high dose was reduced to 200 mg/kg/day beginning on Day 10 due to one female who was sacrificed *in extremis* at 250 mg/kg/day.

No adverse, treatment-related effects were observed on ophthalmoscopic examinations, urinalysis, organ weights or gross or microscopic pathology.

Mortality was observed at high-dose level. One female was sacrificed *in extremis* due to abnormal excreta, dermal atonia, emesis, excessive salivation, hypoactivity and thinness. Treatment-related clinical observations consisted of emesis, diarrhea, soft feces and some mucoid feces in the mid- and high-dose males and females. Treatment-related effects on body weights were observed at high-dose level in both sexes. Males at this dose actually lost weight ($p<0.05$) during Weeks 0-1 and 0-2. Cumulative body weight gains in the males at this dose remained decreased compared to controls throughout the study, and attained statistical significance ($p<0.05$). Overall (Weeks 0-13) body weight gain in high-dose males was decreased compared

to controls. As a result of these decreases, body weights were decreased (non-significant) in these males throughout the study. Treatment-related effects in the high-dose females were limited to decreases (non-significant) in body weight during Weeks 1 and 2. All other statistically significant differences from controls in body weight or body weight gain were minor, transient, and/or unrelated to dose. Red blood cell parameters (red cell count, hemoglobin, and hematocrit) were decreased in males and females at both testing intervals (non-significant). This change was not dose-dependent, but the high-dose groups consistently showed the greatest decrease, while higher platelet counts were found in this dose group. A dose-response decrease in serum albumin and total protein concentrations was observed in the high-dose males and females ($p \leq 0.01$). Serum albumin concentration was decreased in the low-dose males and total protein concentration was decreased ($p \leq 0.05$) in the low-dose females. Serum calcium, which is predominantly bound to albumin, was also decreased ($p < 0.01$ or NS) in the high-dose males and females, but the effect in females was only observed during week 7. Triglycerides were increased or decreased in all treated males, but this effect was not dose-dependent.

The LOAEL is 75 mg/kg/day, based on treatment-related clinical findings in both sexes and dose-response decreases in albumin (males) and total protein concentrations (females). The NOAEL is 25 mg/kg/day.

This study is classified **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3150; OECD 409) for a subchronic oral toxicity study in dogs.

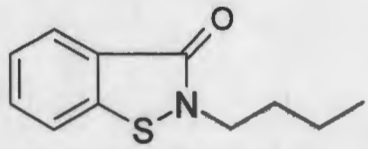
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material(s):** N-butyl-1,2-benzisothiazolin-3-one (BBIT)

Description:	Thick, clear, amber liquid
Lot No.:	6180
Purity:	99.4% a.i.
Stability:	Stable in corn oil for 7 days at room temperature
CAS #:	4299-07-4
Structure:	



2. **Vehicle:** **The test material was suspended in** corn oil; animals were dosed via gelatin capsules (size #11, 10 mL).

3. **Test animals**

Species:	Dog
Strain:	Beagle
Age and weight at initiation of treatment:	Approximately 5-6 months; 8.3-11.1 kg males, 6.4-8.6 kg females
Source:	Ridglan Farms (Mt. Horeb, WI)
Housing:	The dogs were housed individually in stainless steel cages, and allowed to exercise in accordance to standard operating procedures.
Diet:	400g of PMI Nutrition International, LLC, Certified Canine LabDiet ® 5007 (Chow) offered once daily
Water:	Reverse osmosis-treated drinking water, *ad libitum*
Environmental conditions	
Temperature:	20.1-21.4 °C
Humidity:	37.2-58.2%
Air changes:	≥10/hour
Photoperiod:	12 h light/12 h dark
Acclimation period:	14 days

B. STUDY DESIGN

1. **In life dates:** Start: 02/28/06 End: 05/31/06

2. **Animal assignment:** The dogs were randomly allocated (stratified by body weight in a block design) to the groups shown in Table 1.

TABLE 1: Study design [a]			
Test group	**Dose group (mg/kg/day)**	**# Males**	**# Females**
Control	0	4	4
Low dose	25	4	4
Mid dose	75	4	4
High dose	250/200[b]	4	4

a Data were obtained from page 19 of the study report.
b The high dose was reduced from 250 to 200 mg/kg/day beginning on Day 10.

3. **Dose-selection rationale:** It was stated that the doses used for this study were selected based on the results of a dietary rat study that resulted in gastrointestinal effects and lower body weights at an average dose level of 160 mg/kg/day (no further details provided). A dose range-finding study (WIL-544005) at doses of 25, 75 and 175 mg/kg/day in capsules showed some minor clinical findings consisting of soft feces, diarrhea and emesis at the 75 and 175 mg/kg/day dose levels. There were no effects in food consumption values and only variable and transient changes in body weight. Clinical pathology evaluation showed subtle changes without any microscopic histopathological correlates. Consequently the high dose was increased to 250 mg/kg/day for the current study.

4. **Dose preparation, administration and analysis:** Dose formulations were prepared weekly by mixing an appropriate amount of the test article along with vehicle into calibrated containers and mixing until uniform. The dosing concentrations used were 50, 150 and 500/400 mg/mL. The test article formulations were stirred continuously throughout preparation and sampling, and using a syringe the required volume of the correct test substance-corn oil mixture was placed in a gelatin capsule (size #11, 10 mL) immediately prior to dosing for each individual dog. Individual doses were adjusted weekly based on the most recently recorded body weights. The time of day for dosing was not reported. On day 10 of the study one female in the 250 mg/kg/day dose group level was euthanized in extremis. As a consequence, the high dose level was lowered to 200 mg/kg/day beginning on study day 10. Stability analyses were performed on the 50 mg/mL and 500 mg/mL formulations after room temperature storage for up to 11 days. Homogeneity (top, middle, bottom) analyses were performed on samples from the 50 and 500 mg/mL formulations prior to initiation of dosing. Concentration analyses were performed on all dose levels from the first four formulations used in the study and the first 400 mg/mL formulation.

Results

Homogeneity (%RSD): 1.4%

Stability (% of initial): 96.3-97.8% following room temperature storage for 11 days

Concentration (% of nominal): 95.4-105% for the 50, 150, 500 and 400 mg/mL formulations in corn oil

5. **Statistics:** Significance was reported at $p<0.01$ or 0.05. The following parameters were subjected to statistical analysis: body weights, body weight gains, food consumption, clinical pathology and organ weight data were subjected to a parametric 1-way analysis of variance (ANOVA). If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the test article-treated groups to the control group.

   The statistical analyses were considered appropriate.

C. METHODS

1. Observations

a. **Clinical observations:** The dogs were observed at least twice daily for viability, as well as at time of dosing. Cageside observations were recorded on the day prior to study initiation, and then 20-30 minutes and 2 hours post-dosing during treatment. A detailed physical examination of each dog was performed weekly, beginning two weeks prior to study initiation.

b. **Neurological evaluations:** Neurological examinations were not conducted.

2. **Body weight:** Individual body weights were recorded weekly, beginning approximately 2 weeks prior to test article administration. Mean body weights and mean body weight changes were calculated for the corresponding intervals. Final body weights (fasted) were recorded prior to each scheduled necropsy.

3. **Food consumption:** Food consumption was measured daily starting two weeks prior to study initiation and continuing through study termination.

4. **Ophthalmoscopic examination:** An indirect ophthalmoscopic examination was performed on all dogs two weeks prior to study initiation and during Week 12.

5. **Hematology and clinical chemistry:** Blood samples were collected from the jugular vein of overnight fasted dogs prior to initiation of treatment and during Weeks 7 and 12. The following CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*	X	Erythrocyte morphology
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus*	X	Total cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg.*)	X	Total bilirubin*
X	Alkaline phosphatase (ALP)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	Albumin/globulin ratio
X	Alanine aminotransferase (ALT; SGPT)*		
X	Aspartate aminotransferase (AST; SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

6. **Urinalysis:** Urinalysis was performed prior to initiation of treatment and during Weeks 7 and 12 of dosing. Samples were collected from individually housed dogs using cage pans for urine collection. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin
X	pH*	X	Blood*
X	Sediment (microscopic)	X	Nitrite
X	Protein*	X	Urobilinogen
X	Color	X	Leukocytes

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

7. **Sacrifice and pathology:** All animals were euthanized by exsanguination while under sodium pentobarbital anesthesia, and subjected to a gross necropsy. The CHECKED (X) tissues were collected for microscopic examination. Additionally, the (XX) organs were weighed from all animals. Paired organs were weighed together.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic*	XX	Brain* +
X	Salivary glands*	XX	Heart* +	X	Peripheral nerve (sciatic)*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen* +	X	Eyes (with optic nerves)*
X	Jejunum*	XX	Thymus* +		GLANDULAR
X	Ileum*			XX	Adrenal gland* +
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys* +	XX	Parathyroid* + [a]
X	Rectum*	X	Urinary bladder*	XX	Thyroid* + [a]
XX	Liver* +[b]	XX	Testes* +		OTHER
XX	Gall bladder* +[b]	XX	Epididymides* +	X	Bone (femur)
X	Pancreas*	X	Prostate*	X	Bone (Sternum)
	RESPIRATORY	XX	Ovaries* +	X	Skeletal muscle
X	Trachea*	X	Oviducts	X	Skin*
X	Lungs*	XX	Uterus* +	X	All gross lesions and masses*
X	Nasal cavity*	X	Mammary area*		
X	Pharynx*	X	Cervix		
X	Larynx*	X	Ureters		
		X	Vagina		

a The parathyroids were weighed with the thyroid.
b The liver was weighed with the gall bladder
* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150
+ Organ weight required for non-rodent studies.

All tissues were preserved in neutral-buffered 10% formalin, except the epididymides, testes and eyes. The epididymides and testes were fixed in Bouin's Solution; the eyes were fixed in Davidson's Solution. Tissue samples were processed routinely and stained with hematoxylin and eosin. All listed tissues from all dogs were examined microscopically. Microscopic pathological findings were graded as minimal, slight/mild, moderate, moderately severe, or severe/high.

II. RESULTS: The high dose was reduced from 250 mg/kg/day to 200 mg/kg/day on Day 10. This group will be referred to as the 250/200 mg/kg/day group throughout the results.

A. OBSERVATIONS

1. **Mortality:** One female (#1890) in the 250 mg/kg/day group was euthanized in extremis on study day 10. Clinical findings prior to euthanasia included abnormal excreta, dermal atonia, emesis, excessive salivation, hypoactivity and thinness. Additionally, food consumption for this female was severely reduced and an attempt was made to stimulate her appetite by supplemental feeding. This female had lost 14% of her study day 0 body weight by study day 10. As a consequence of this female's response to the testing material and the clinical findings (diarrhea and emesis) in other surviving males and females in the 250/mg/kg/day dose group, the dosage level was lowered from 250 to 200 mg/kg/day on study day 10. All other dogs survived to the scheduled termination.

2. **Clinical signs of toxicity:** Treatment-related clinical observations consisted of emesis, diarrhea, soft feces and some mucoid feces (some red or yellow in color) in the 75 and 250/200 mg/kg/day group males and females. Emesis and emesis-related findings (containing food, white, yellow or red material or capsule material) were the most prominent clinical observations and were noted in the highest incidence at the 20-30 minute post dosing in the 75 and 250/200 mg/kg/day group males and females. The incidence of soft feces and diarrhea was higher in the 75 and 250/200 mg/kg/day group males and females at both the 20-30 minute and 2-hour post-dose observations. At the 20-30 minute post dosing, a slightly higher incidence of mucoid feces was found in the 75 and 250/200 mg/kg/day males but was sporadically observed in the females. Similar pattern was seen at the 2-hour post dosing.

B. **BODY WEIGHT AND WEIGHT GAIN:** Selected body weight and body weight gain data are presented in Table 2. Treatment-related effects on body weights were observed at 250/200 mg/kg/day in both sexes. Males at this dose actually lost weight ($p<0.05$) during Weeks 0-1 and 0-2 (-0.1 kg each treated vs. 0.5 to 0.7 kg controls). Cumulative body weight gains in the males at this dose remained decreased by 55-90% compared to controls throughout the study, and attained statistical significance ($p<0.05$) during Weeks 0-3, 0-6 ($p<0.01$), 0-8, 0-9, and 0-10. Overall (Weeks 0-13) body weight gain in the 250/200 mg/kg/day males was decreased by 50% compared to controls. As a result of these decreases, body weights were decreased (NS) by 9-16% in these males throughout the study. Treatment-related effects in the 250/200 mg/kg/day females were limited to decreases (NS) in body weight of 10-12% compared to controls throughout the study. All other statistically significant differences from controls in body weight or body weight gain were minor, transient, and/or unrelated to dose.

TABLE 2. Selected group mean (±SD) body weights and cumulative body weight gains (kg) in dogs administered BBIT in the diet for up to 13 weeks.[a]

Study week	Dose (mg/kg/day)			
	0	25	75	250/200[b]
Males				
0	9.7 ± 1.05	9.5 ± 1.12	9.7 ± 0.65	9.4 ± 0.82
1	10.2 ± 0.97	9.9 ± 1.07	10.1 ± 0.68	9.3 ± 0.90 (↓9%)
13	12.9 ± 1.64	12.0 ± 1.39	12.4 ± 0.79	10.9 ± 1.08 (↓16%)
BWG Weeks 0-1	0.5 ± 0.21	0.4 ± 0.19	0.4 ± 0.22	-0.1 ± 0.34* (↓80%)
BWG Weeks 0-2	0.7 ± 0.40	0.6 ± 0.50	0.7 ± 0.10	-0.1 ± 0.45* (↓86%)
BWG Weeks 0-3	1.0 ± 0.48	0.9 ± 0.43	1.0 ± 0.19	0.1 ± 0.48* (↓90%)
BWG Weeks 0-12	3.3 ± 1.33	2.4 ± 0.96	3.0 ± 0.22	1.5 ± 0.79 (↓55%)
BWG Weeks 0-13	3.2 ± 1.14	2.5 ± 1.02	2.7 ± 0.49	1.6 ± 0.66 (↓50%)
Females				
0	7.5 ± 0.91	7.4 ± 0.71	7.4 ± 0.94	7.2 ± 0.77
1	7.8 ± 1.18	7.7 ± 0.58	7.5 ± 0.92	7.0 ± 0.53 (↓10%)
2	7.8 ± 1.02	7.8 ± 0.43	7.6 ± 0.84	6.9 ± 0.69 (↓12%)
13	9.7 ± 1.24	9.5 ± 0.29 (↓2%)	8.7 ± 0.80 (↓10%)	8.8 ± 0.35 (↓10%)
BWG Weeks 0-13	2.2 ± 0.61	2.1 ± 0.87	1.4 ± 0.29	1.9 ± 0.44 (↓14%)

a Data were obtained from Tables 6 through 8 on pages 98-117 of the study report. BWG = Body weight gain. Percent differences from controls are included in parentheses. n=4 except for the 250/200 mg/kg/day females, where n=3.

b Dogs were administered the test compound at 250 mg/kg/day on Days 0-10, then at 200 mg/kg/day on Days 11-91.

* Significantly different from controls; $p<0.05$

C. **FOOD CONSUMPTION:** In 250/200 mg/kg/day males, food consumption was decreased ($p<0.05$) by 27% during the first week of dosing. Mean food consumption remained decreased (NS) by 17% compared to controls in the males during Week 1-2 and was also decreased by 15-19% in the 250/200 mg/kg/day females during Weeks 0-1 and 1-2. Food consumption was unaffected by treatment at 25 and 75 mg/kg/day in both sexes.

D. **COMPOUND INTAKE:** The actual dosages of the test material to the dogs are presented in Table 1.

E. **OPHTHALMOSCOPIC EXAMINATION:** There were no treatment-related findings observed during the ophthalmoscopic examinations.

F. **BLOOD ANALYSES**

1. **Hematology:** Selected hematology parameters are presented in Table 3 below. In the 250/200 mg/kg/day males, decreases in red blood cells (↓9%), hemoglobin (↓12-17%), and hematocrit (↓12-16%) were observed during Week 7 and 12. Although these decreases were not statistically significant, the study researcher(s) considered them to be treatment-related, based on the consistent trends between sexes, the history of red material in the feces and/or emesis, and the magnitude of decreases in individual animal compared to historical controls (data not provided). These observed hematological changes were considered treatment-related effects also due to the fact that the tendency of these changes was continually to decrease with time and consistently remained lower than the pretest values, rather than the observation seen in the other treatment and control groups that these parameters would increase relatively to their respective pretest values at Week 7 and either remain higher than or closely approximate to the pretest values by Week 12. Additionally, platelet counts were increased ($p<0.05$) by 43-60% in the 250/200 mg/kg/day males during Weeks 7 and 12. No treatment-related effects on hematology parameters were observed in the females at any dose or in the males at 25 or 75 mg/kg/day. All other statistically significant differences from controls noted were minor and/or unrelated to treatment.

TABLE 3. Selected mean (±SD) hematology parameters in male dogs administered BBIT in the diet for up to 13 weeks.[a]

Observation	Week	Dose (mg/kg/day)			
		0	25	75	250/200[b]
RBC (mil/uL)	-2	6.88±0.53	6.59±0.19	6.78±0.14	6.78±0.28
	7	7.14±0.76	6.79±0.49	6.90±0.47	6.51±0.66 (↓9%)
	12	7.07±0.38	6.53±0.46	6.56±0.46	6.44±0.70 (↓9%)
Hemoglobin (g/dL)	-2	15.7±1.05	15.3±0.56	15.3±0.64	15.2±1.17
	7	16.3±1.65	16.1±1.15	16.1±1.39	14.4±1.02 (↓12%)
	12	16.3±0.75	15.6±1.14	15.4±0.99	13.6±1.92 (↓17%)
Hematocrit (%)	-2	44.9±2.93	44.2±1.92	44.5±2.09	43.1±3.02
	7	46.6±4.21	46.2±3.56	45.6±3.76	41.0±2.88 (↓12%)
	12	45.9±2.26	44.2±2.94	43.4±2.64	38.7±5.09 (↓16%)
Platelets (thous/uL)	-2	460±63.2	469±114.1	470±124.8	519±124.3
	7	333±48.7	313±82.8	364±88.3	475±60.3* (↑43%)
	12	310±28.6	318±76.7	354±82.8	497±45.1** (↑60%)

a Data were obtained from Table10 on pages 124-126, 129, and 147 of the study report. Percent differences from controls are included in parentheses, n=4.

b Dogs were administered the test compound at 250 mg/kg/day on Days 0-10, then at 200 mg/kg/day on Days 11-91.

* Significantly different from controls; $p<0.05$

** Significantly different from controls; $p<0.01$

2. **Clinical chemistry:** Serum albumin and total protein concentrations were decreased ($p<0.01$) by 22-32% and 18-27%, respectively, in the 250/200 mg/kg/day males and females. Serum albumin concentration was decreased by 8-11% ($p\leq0.05$) in the 75 mg/kg/day males and total protein concentration was decreased by 9% ($p\leq0.05$) in the 75 mg/kg/day females. Such decreases in serum albumin and total protein concentrations were seen in a dose-response manner. As a result, the albumin/globulin ratio was also decreased in the 250/200 mg/kg/day males. Serum calcium, which is predominantly bound to albumin, was also decreased 6.1-9.9% ($p<0.01$ or NS) in the 250/200 mg/kg/day males and females. Triglycerides were increased ($p<0.01$) in the 75 and 250/200 mg/kg/day males during Weeks 7 and 12, but it was only considered to be treatment-related at week 7 due to a lack of dose-dependent relationship for this effect during Week 12. All other statistically significant differences from controls were minor, sporadic, and/or not related to dose.

TABLE 4. Selected group mean (±SD) clinical chemistry in dogs administered BBIT in the diet for up to 13 weeks.[a]				
Study week	Dose (mg/kg/day)			
	0	25	75	250/200[b]
Males				
Albumin				
-2	3.6 ± 0.21	3.5 ± 0.13	3.6 ± 0.17	3.5± 0.16
7	3.7 ± 0.17	3.7 ± 0.13	3.4 ± 0.13(↓8%)*	2.5 ± 0.08 (↓32%)**
12	3.7 ± 0.18	3.6 ± 0.06	3.3 ± 0.17 (↓11%)*	2.5 ± 0.24 (↓32%)**
Calcium				
-2	11.6 ± 0.21	11.6 ± 0.42	11.9 ± 0.59	11.7 ± 0.33
7	11.5 ± 0.21	11.5 ± 0.32	11.1 ± 0.33	10.5 ± 0.22(↓8.7%)**
12	11.1 ± 0.21	11.0 ± 0.33	10.7 ± 0.22	10.0 ± 0.24 (↓9.9%)
Total Protein				
-2	5.5 ± 0.25	5.6 ± 0.24	5.7 ± 0.21	5.6 ± 0.34
7	5.7 ± 0.13	5.7 ± 0.29	5.4 ± 0.33	4.4 ± 0.08 (↓23%)**
12	5.6 ± 0.33	5.5 ± 0.18	5.2 ± 0.32	4.1 ± 0.29 (↓27%)**
Triglyceride				
-2	27 ± 1.5	29 ± 4.0	26 ± 2.9	27 ± 5.4
7	26 ± 3.4	29 ± 4.5	39 ± 5.2** (↑50%)	43 ± 5.0** (↑65%)
12	24 ± 9.1	34 ± 11.3	26 ± 3.3	30 ± 7.9 (↑25%)
Females				
Albumin				
-2	3.4 ± 0.18	3.3 ± 0.17	3.4 ± 0.05	3.5 ± 0.10
7	3.7 ± 0.10	3.5 ± 0.22	3.4 ± 0.10 (↓8%)	2.9 ± 0.25 (↓22%)**
12	3.7 ± 0.24	3.5 ± 0.41	3.4 ± 0.08(↓8%)	2.7 ±0.15 (↓27%)**
Calcium				
-2	11.7 ± 0.25	11.5 ± 0.37	11.7 ± 0.16	11.7 ± 0.10
7	11.4 ± 0.22	11.2 ± 0.35	11.3 ± 0.25	10.7 ± 0.15(↓6.1%) **
12	11.0 ± 0.28	10.8 ± 0.57	10.8 ± 0.21	10.3 ± 0.17 (↓6.4%)
Total Protein				
-2	5.4 ± 0.39	5.1 ± 0.37	5.1 ± 0.14	5.7 ± 0.34
7	5.7 ± 0.14	5.3 ± 0.22 (↓7%)*	5.2 ± 0.13 (↓9%)*	4.7 ± 0.26 (↓18%)**
12	5.5 ± 0.22	5.1 ± 0.33	5.1 ± 0.19	4.3 ± 0.35 (↓22%)**

a Data were obtained from Table 11 on pages 160-189 of the study report. Percent differences from controls are included in parentheses. n=4 except for the 250/200 mg/kg/day females, where n=3.

b Dogs were administered the test compound at 250 mg/kg/day on Days 0-10, then at 200 mg/kg/day on Days 11-91.

* Significantly different from controls; $p<0.05$

** Significantly different from controls; $p<0.01$

G. **URINALYSIS:** There were no treatment related effects observed on the measured urinalysis parameters. A. statistically significant increase in specific gravity was noted in the 250/200 mg/kg/day females during Week 7; however, these animals began with a higher average specific gravity (Week -2) and individual data show conflicting time-related responses. Therefore, this effect is considered unrelated to treatment.

H. SACRIFICE AND PATHOLOGY

1. **Organ weight:** There were no adverse, treatment-related effects observed on organ weights. At 250/200 mg/kg/day, adrenal gland weights were increased by 16-17% in both males and females ($p<0.05$), however, in the absence of a histological correlation for the weight increase, the toxicological significance of this change is uncertain. Additionally, due to the age, variations in sexual maturity and the absence of histological alterations in the uterus, the toxicological significance of lower uterine weights (↓53%) in the 250/200

g/kg/day group is uncertain and likely driven by one of the three females with an extremely low uterine weight. No other differences ($p<0.05$) were observed.

2. **Gross pathology:** Red discoloration within the gastrointestinal tract in the 250 mg/kg/day female euthanized *in extremis* was considered treatment related. Although this finding was a common necropsy observation at all doses, especially in the 250/200 mg/kg/day group males and females, the Sponsor considered to be test article-related in the 250/200 mg/kg/day group only. All other gross pathological findings were observed in a single animal and/or were not dose-dependent.

3. **Microscopic pathology:** Definitive treatment related changes were limited to, the gastrointestinal tract of the female euthanized on Day 10. This animal had minimal to moderate necrosis, often with hemorrhage and/or thrombosis, in the larynx, pharynx, stomach, jejunum, ileum, cecum, colon and rectum. These changes likely resulted from direct irritation of the test article as it passed through the digestive tract. No other treatment related changes were observed. In the remaining dogs, the only histological finding of interest was goblet cell hyperplasia in the colon of a single 250/200 mg/kg/day male. This change, if related to treatment is an adaptive response to local irritation. All other microscopic observations were considered spontaneous, incidental, and/or unrelated to dose.

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** Administration of the test substance at a dose of 250 mg/kg/day for 10 days followed by administration at 200 mg/kg/day for the remainder of the study resulted in treatment-related effects, including emesis, diarrhea, soft and mucoid feces, lower body weights and food consumption, lower red blood cell parameters, higher platelet counts and lower albumin and total protein concentrations. The NOAEL was 75 mg/kg/day. At this dose level clinical findings consisted of emesis and emesis related findings, diarrhea and soft and mucoid feces. These findings were not accompanied by any microscopic or clinical pathologic correlates or effects on body weights.

B. **REVIEWERS' COMMENTS:** No adverse, treatment-related effects were observed on ophthalmoscopic examinations, urinalysis, organ weights or gross or microscopic pathology.

Mortality was observed at 250/200 mg/kg/day. One female was sacrificed *in extremis* due to abnormal excreta, dermal atonia, emesis, excessive salivation, hypoactivity and thinness. Treatment-related clinical observations consisted of emesis, diarrhea, soft feces and some mucoid feces (some red or yellow in color) in the 75 and 250/200 mg/kg/day group males and females. Treatment-related effects on body weights were observed at 250/200 mg/kg/day in both sexes. Males at this dose actually lost weight ($p<0.05$) during Weeks 0-1 and 0-2 (-0.1 kg each treated vs. 0.5 to 0.7 kg controls). Cumulative body weight gains in the males at this dose remained decreased by 55-90% compared to controls throughout the study, and attained statistical significance ($p<0.05$) during Weeks 0-3, 0-6, 0-8, 0-9, and 0-10. Overall (Weeks 0-13) body weight gain in the 250/200 mg/kg/day males was decreased by 50% compared to controls. As a result of these decreases, body weights were decreased

(NS) by 9-16% in these males throughout the study. Treatment-related effects in the 250/200 mg/kg/day females were limited to decreases (NS) in body weight of 10-12% compared to controls during Weeks 1 and 2. All other statistically significant differences from controls in body weight or body weight gain were minor, transient, and/or unrelated to dose. Red blood cell parameters were decreased in males and females at both testing intervals (NS). This change was not dose-dependent, but the 250/200 mg/kg/day dose groups consistently showed the greatest decrease. A dose-response decrease in serum albumin and total protein concentrations were observed in the 250/200 mg/kg/day males and females (22-32% and 18-27%, respectively; $p \leq 0.01$). Serum albumin concentration was decreased by 8-11% ($p \leq 0.05$) in the 75 mg/kg/day males and total protein concentration was decreased by 9% ($p \leq 0.05$) in the 75 mg/kg/day females. Serum calcium, which is predominantly bound to albumin, was also decreased 6.1-9.9% ($p < 0.01$ or NS) in the 250/200 mg/kg/day males and females, but the effect in females was only observed during week 7. Triglycerides were increased or decreased in all treated males, but this effect was not dose-dependent.

The LOAEL is 75 mg/kg/day, based on treatment-related clinical findings in both sexes and dose-response decreases in albumin (males) and total protein concentrations (females). The NOAEL is 25 mg/kg/day.

This study is classified **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3150; OECD 409) for a subchronic oral toxicity study in dogs.

C. **STUDY DEFICIENCIES:** The following minor deficiency was noted, but does not alter the conclusions of this DER: historical control data were not provided.